Liposidolide A, a New Antifungal Macrolide Antibiotic

Sir:

In the course of our screening of new antibiotics, several antifungal macrolide antibiotics have been isolated previously.[1~4] In this communication, we report isolation, physico-chemical properties, structure and biological activity of a new antibiotic, liposidolide A, which was isolated from a culture of *Streptomyces* sp. RS-28. The producing strain was isolated from a soil sample collected in Fijian Province, China.

The strain was cultivated at 27°C for 37 hours in a jar fermenter containing 18 liters of a medium composed of 2% glucose, 1% soluble starch, 0.1% meat extract, 2.5% soybean flour, 0.2% NaCl and 0.005% K_2HPO_4. The fermentation broth (18 liters) was filtered and the mycelial cake was extracted with 80% acetone. The acetone extract was concentrated to give an aqueous solution which was then extracted with buthanol. The buthanol extract was concentarted *in vacuo* to dryness. The residue was dissolved in 50% methanol and adsorbed on Diaion HP-20 column. The column was eluted successively with 50%, 70% and 100% methanol, and the 100% methanol fraction was concentrated *in vacuo* to dryness. The residue was purified by preparative HPLC (Nucleosil $5C_{18}$, 85% (acetonitrile - methanol = 5 : 3), 15% H_2O and additional 0.15% HCOOH, as solvent). The fractions containing liposidolide A were combined and concentrated *in vacuo* to dryness. The active residue was finally purified by HPLC (78% (acetonitrile - methanol = 5 : 3), 22% H_2O and additional 0.15% HCOOH, as solvent). The single peak fraction containing liposidolide A was concentrated to dryness. The residue was liophilized to give a pure powder of liposidolide A (7 mg). It is a colorless powder with a melting point of 126~128°C. It is optically active, with $[\alpha]_D^{20}$ +35.4° (*c* 0.5, MeOH). The molecular formula was determined to be $C_{78}H_{138}O_{30}$ by high resolution FAB-MS *m/z* 1577.9150 $(M+Na)^+$; calcd for $C_{78}H_{138}O_{30}\cdot Na$ 1577.9170, and elementary analysis ; calcd for $C_{78}H_{138}O_{30}\cdot H_2O$; C 59.54, H 8.77%; found C 59.01, H 8.67%. It is easily soluble in methanol, buthanol, hardly soluble in water, acetone, acetonitrile and insoluble in chloroform and ether. It shows positive reaction to Lemieux, iodine vapour and anisaldehyde-H_2SO_4 tests, but negative to ninhydrin, anthrone and ferric chloride tests. The UV spectrum showed an end absorption. The IR spectrum is shown in Fig. 1. The ^{13}C NMR spectrum is shown in Fig. 2, which accounts for the presence of 76 carbon signals (two overlapped signals are present). The structure of liposidolide A was determined by spectroscopic evidences, especially NMR studies and analysis of FAB-MS fragmentations. In the FAB-MS spectrum, characteristic fragment ions *m/z* 1287 (M^+-fatty acid unit), 1143 (M^+-fatty acid and 2,6-deoxysugar units), 981 (1143-mannose), 911 (M^+-side chain portion *e.g.* cleavaged between C-34 and C-35 position) were observed. All ^1H and ^{13}C NMR signals of liposidolide A were completely assigned by careful analyses of 2D NMR spectra including DQF-COSY, TOCSY, NOESY, HMQC, HMQC-TOCSY and HMBC using pulsed field gradient (PFG),[5~7] and detailed experimental data of 1D HOHAHA spectra. Based on the NMR data, the presence of (6*E*)-3,5-dihydroxyhexadec-6-enoic acid, 2,6-dideoxy-4-*O*-methy-*β*-*arabino*-hexopyranoside and α-mannose units were elucidated. Analysis of HMBC data revealed the connectivities inter units described above and intra units of 36-membered macrolide portion. The structure of liposidolide A is an unique 36-membered macrolide with two sugar units and fatty acid as shown in Fig. 3. In view of the structural homology, liposidolide A have some similarilties to 32-membered macrolide notonesomycin A.[8] Details of the structural determination of liposidolide A will be reported in a separated paper. Liposidolide A was inactive against Gram-posi-

Fig. 1. IR spectrum of liposidolide A.

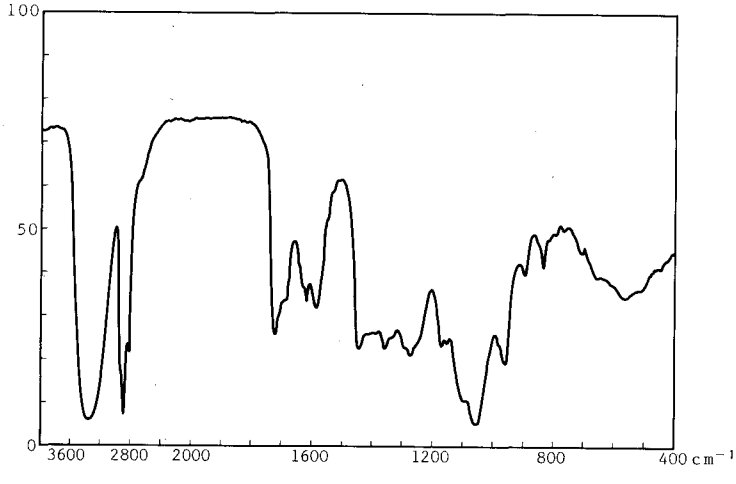
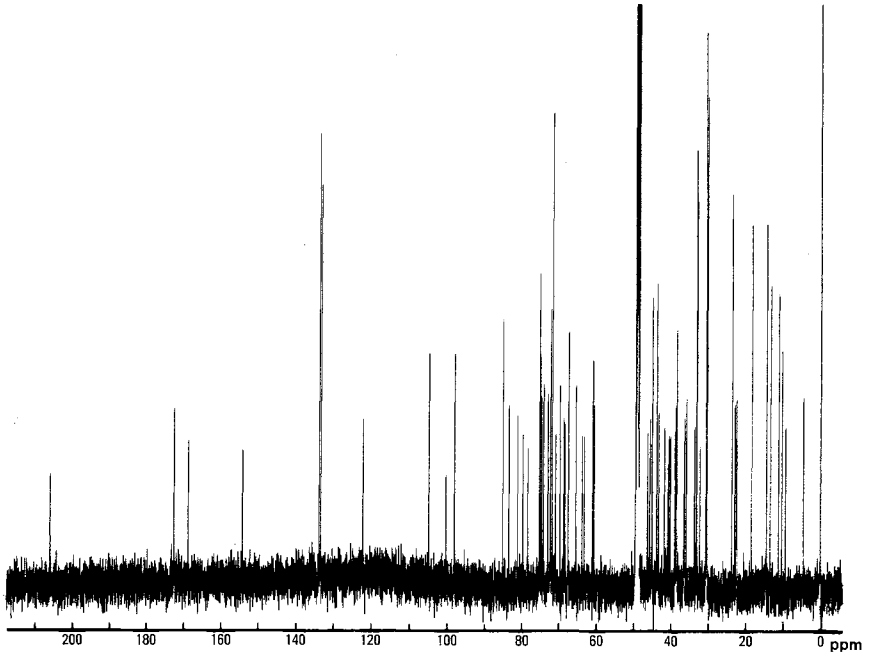


Fig. 2. ^{13}C NMR spectrum of liposidolide A.



150 MHz, $CDCl_3$, TMS as an internal standard.

Fig. 3. Structure of liposidolide A.

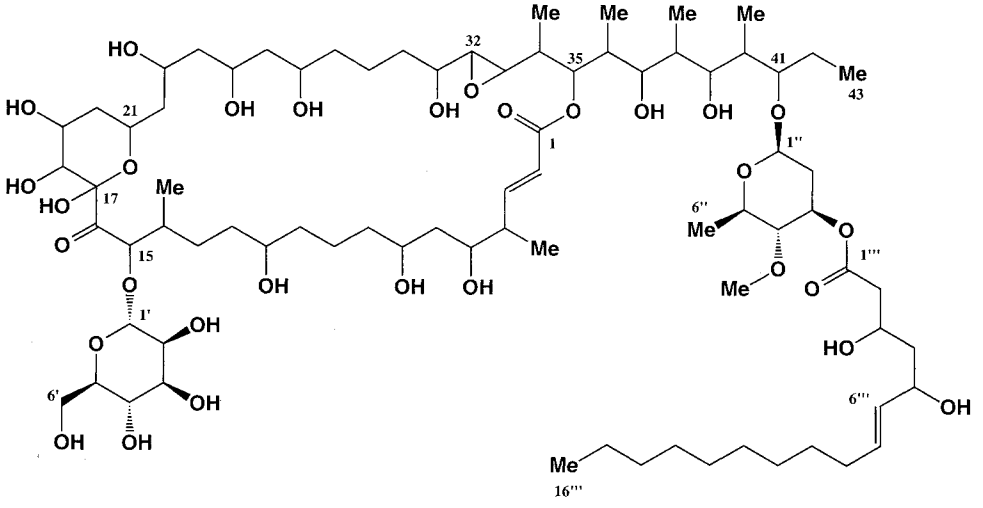


Table 1. Antimicrobial activity of liposidolide A.

Test organism	MIC (μg/ml)
Pyricularia oryzae IFO5994	0.2
Botryotinia fuckeliana IFO5365	3.1
Colletotrichum lagenarium IFO7513	0.1
Candida albicans IFO1594	1.9
Chlorella vulgaris	0.2

* Conventional agar dilutiion method was employed.

tive and Gram-negative bacteria but showed inhibitory activity against some species of phytophathogenic fungi and algae as shown in Table 1. In pot test, it showed a preventive value of 99.4% against cucumber anthracnose at the dose of 50 ppm. LD_{50} to mice was approximately 50 mg/kg by intraperitoneal administration.

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